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# Speeding up scientific illustrations. A method to avoid time consuming pencil drawings particularly in arthropods

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((running head title: Drawing method))

## 1. Abstract

The most time consuming part of a taxonomic description is to make the illustrations. This contribution shows how to save time by omitting the pencil drawings of arthropod appendages and replacing them by microphotographies. These are imported into a drawing software on a computer for making a publication ready line drawing. The photographic method requires a special treatment of the appendages which is also shown.

Keywords: method, drawing, taxonomy, arthropods, microphotography,

## 2. Introduction

The high number of undescribed species and the limited time available for research brings the taxonomist on the horn of a dilemma: describe fewer species, very detailed or describe more species, but not so detailed. Of course most of the taxonomists would agree to deliver high quality work that would be still useful even in the far future as some of our predecessors showed in an exemplary manner (e.g. Stebbing, 1888; Sars, 1889).

The only way out of the dilemma is to speed up the descriptive process. One possibility is the use of the DELTA software (Dallwitz *et al.*, 1993; Dallwitz *et al.*, 1998) to quickly generate text descriptions, diagnoses and keys.

However, the most time consuming part of taxonomy is making the illustrations. Drawing in taxonomy is a two step process, both very time consuming. At first a pencil drawing is made often using a camera lucida. Then these drawings are traced another time on plates for the publication. This inking can be sped up by using digital drawing methods (Coleman, 2003).

The idea of this paper is to combine the quick microphotography and the advantages

of a scientific drawing. Moreover, this method is very cost-effective, compared to the purchase of a camera lucida, which for some microscopes is already difficult to buy. This method is especially good for dealing with arthropod appendages. These have to be treated in a special way prior to digitization: muscles must be dissolved, the cuticle decalcified and stained. Then the appendages are mounted on microscopic slides.

### 3. Material and Methods and step by step guide

#### 3.1 Preparation and making of permanent slides

The prerequisite for the method is to clear the appendages by maceration and decalcification, staining and mounting them before taking the photos.

**Maceration.** There are two ways of dissolving the muscles inside the appendages.

1) One can use an enzyme solution. I used two different recipes: a) 3 ml sodium-borate solution in 7 ml aqua dest. with 0.1 g trypsin; b) enzyprim solution: NaCl 15 g, Enzyprim 20 ml; Mollecal C 0.5 g in 1 l aqua dest., adjust to pH 8.5-9 with  $\text{NaCO}_3$ .

The appendages are dissected off the body and put in a small vial with a glass lid. Prior to the enzyme treatment the body parts should be watered to remove ethanol or formaldehyde solution. The vial should be put in a warm environment, e.g. a hotplate, at temperatures of 40-45 °C (Fig. 1a). Check the vials often and replace any evaporated water. The time required for dissolving the tissue varies from 1-6 hours depending on the size of the objects.

2) I prefer the KOH maceration. The appendages are put in a staining block with 70% ethanol and 1-2 pellets of KOH are added. Also add 1 ml of glycerol, which prevents that the objects fall dry during the further processing. After heating on a

hot plate (not more than 50-60 °C) the KOH dissolves quickly. Add ethanol from time to time to fill up liquid losses. After 2-3 hours the tissue is completely dissolved. Put appendages into fresh glycerol.

**Decalcification:** When the cuticle is not completely transparent it may be encrusted with calcium carbonate. The calcium carbonate can be eliminated easily by putting the appendages from glycerol into 70% ethanol in which some crystals of citric acid were dissolved. Rests of glycerol prevent gas bubbles from forming inside the appendages.

**Staining:** After the maceration process the body parts are transferred into a fresh staining block with 70% ethanol and add some crystals of “lignin-pink” (Azophloxin,  $C_{18}H_{13}N_3Na_2O_8S_2$ ). Add 1 ml of glycerol and heat on a hotplate (Fig. 1a). Visually control the staining and stop the process when you are content. Then transfer the appendages into fresh glycerol.

**Making of permanent slides:** Two mounting media are recommendable:

Canadabalsam and Euparal. Both are very lasting and there are slides mounted with these media that are more than 100 years old and still are in excellent condition.

Canadabalsam is rather difficult to handle and some components are very toxic. Thus I prefer Euparal (Chroma, 3C 240 green). In order to allow the medium to penetrate easier, I put the appendages into a mixture of one part 70% ethanol and one part Euparal. After an hour the objects are transferred into pure Euparal for at least another hour before mounting them onto slides.

If only single appendages are mounted on separate slides and the appendages are rather small there are no difficulties. If the appendages are thick then put the appendage into a large droplet of Euparal and let it dry for a while, put another drop on the cover slip you want to use and let it rest (Fig. 1b: arrow). If the resin gets gluey

and tears threads slowly put the cover slip on the object. It may be that the cover slip is raised on one side if the object is very thick, here put some extra Euparal. During the drying process the mounting medium may somewhat contract. Resulting gaps can be filled from the side.

It is also possible to mount several small appendages onto one slide. In order to prevent the appendages from moving around when putting on the cover slip, put a droplet of Euparal on the slide and use a cover slip to tear out the drop. Put the appendages in this layer of Euparal in the wanted order and let them rest until the medium becomes gluey. Then, put another droplet on the cover slip and put on the slide after the resin became gluey. Let the slide dry for some days in a dust free environment before making the micrographs.

### **3.2 Photography**

For making the photos we use a Canon Power Shot A 80 digital camera mounted with an adapter tube on a “Zeiss Axiostar plus” microscope (Fig. 2a: 1). From each article of the appendages several micrographs are made varying the depth of focus. Then the data are transferred onto the computer with a link cable.

The Canon Power Shot camera is delivered with the “Zoom Browser EX” software which allows, among other features, to control all camera settings and transfer the files directly on the hddisk of the computer (remote capture task, Fig. 2c). There is a small preview window, this gives a live picture from the microscope. So there is no need to look through the microscope to control the focus, the whole process can be done on the computer screen. Three settings should be adapted before taking the photo with the remote capture option: 1) adjust the zoom with the lever on the right side of the window (Fig. 2c: 1), so that the dark margins disappear; 2) select in preferences “artificial light” and 3) open the f-stop as much as possible. With this software it is much quicker to make the micrographs compared to the direct

adjustments on the camera. Click on the button up in the right corner of the window (Fig. 2c: 2) to shoot a picture. Make a stack of micrographs of an article of an appendage and save these files in an own subdirectory. Then move to the neighbouring article of the appendage and produce another stack. Repeat until the appendage is completely photographed.

Open the drawing software (e.g. Acrobat Illustrator). Each picture from a stack are transferred on separate layers of a drawing software using the “place” comand (for details see Coleman, 2003). Then add the first picture of the next stack of micrographs and “place” it next to the already placed stack, so that the corresponding parts overlap. Without changing the position put the next pictures from the stack on separate layers. When all picture stacks are combined, it is possible to move through the different focus layers by clicking on the layer selectors (Fig. 2b: arrow). Only one level is shown at a time, there is no addition of all focus levels.

Draw on a newly created layer, put on top of all microphotographies and trace the photographies using a WACOM Intuous 2 DIN A3 drawing tablet (Fig. 2a: 3) (how to do this compare with Coleman, 2003) and select the appropriate focus level with the layer selector. During drawing the permanent slide should be looked at through the microscope in order to avoid mistakes. Only the layer containing the digital drawing is used to arrange the single drawings of the appendages on plates.

## 4. Discussion

The digital drawing method (Coleman, 2003) let the taxonomist speed up the making of line drawings. Moreover, as text processors revolutionized our writing the use of drawing software allows to try until we are satisfied with the result. Selecting special settings in the software perfect lines without any signs of tremble can be made. Small structures can be enlarged dramatically and the drawing of these structures is much easier compared to the traditional inking on paper. Different weights and styles of

lines can be defined and retrieved by macros. With this technique everybody can quickly make perfect line drawings.

But this digital inking is only the second part of the illustration process. The basis for this digital drawing method is a conventional pencil drawing, made with a microscope and a camera lucida. In order to really save time, the pencil drawing must be avoided somehow.

Alan Myers (personal communication) tried to use modern microphotography and combined images of different depth of focus into one photo in order to avoid the time-taking drawing process at all. However, this method has some fundamental disadvantages: 1) muscles inside the appendages sometimes block the view on details on the lower focal plain; 2) it may be difficult to see, if details are on the upper or lower face of the appendage, as the focal planes are condensed into one picture 3) artefacts on the cuticle can not be corrected like in a drawing; 4) it is time consuming to erase disturbing background artefacts in a photography using image processing software.

On the other hand a drawing is much clearer than a micrograph and even small, inconspicuous details, such as fine structures of setae, that are relevant for systematics can be shown. A drawing is not only a documentation of the morphology but also an interpretation, showing the important characters some of which are difficult to see in a photo, e.g. sometimes fine lines between articles of appendages. These cuticular lines are very important for measuring the length of articles, length width ratios are often used as a taxonomic character. Different from a photo, structures that are surpassed by others can be indicated in a drawing by dashed lines. Another advantage of the digital drawing method is that most of the illustration procedure can be made by technical staff. Later the systematist checks the results and if necessary corrects mistakes.

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## Figure captions

Fig. 1a-c. Preparation of permanent slides. a: maceration of appendages on a hot plate removing tissue from the appendages; b: making of permanent slides using Euparal, arrow directs on cover slip with drop of Euparal, resting to allow the mounting medium to becoming somewhat harder; c: lignin-pink stained distal part of a leg of an amphipod crustacean mounted in Euparal.

Fig. 2a-c. Making of micrographs. a: stacks of photos are taken with a camera mounted on a microscope (1) and directly transferred on a computer (2) and drawn with a WACOM Intuous 2 DIN A3 drawing tablet; b: stacks of micrographs from single articles are imported in a drawing software and arranged to one picture; focus levels can be selected by clicking on the level selector (arrow); c: all camera functions and the focus of the microscope are controlled by the “remote capture task” software. Moving the lever “1” up will zoom out the dark margins; pushing the button “2” will expose a picture and automatically transfer it on the computer.



